Astringency sensitivity to tannic acid: Effect of ageing and salivary proline-rich protein levels

Mei Wang, Hélène Brignot, Chantal Septier, Christophe Martin, Francis Canon, Gilles Feron

Food Chemistry: Molecular Sciences 8 (2024) 100192

Contents lists available at ScienceDirect

Food Chemistry: Molecular Sciences

journal homepage: www.sciencedirect.com/journal/food-chemistry-molecular-sciences

Astringency sensitivity to tannic acid: Effect of ageing and salivary proline-rich protein levels

Mei Wang, Hélène Brignot, Chantal Septier, Christophe Martin, Francis Canon *, Gilles Feron *

Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne, F-21000 Dijon, France

ARTICLE INFO

Keywords:
Astringency
Threshold
bPRPs
gPRPs
Elderly
Tannic acid

ABSTRACT

The link between salivary composition and sensitivity to astringency as a function of age has still not been established. In this work, we propose the hypothesis that ageing leads to changes in the concentration of salivary proline-rich proteins (PRPs), which alters the astringency perception threshold with age. To test this hypothesis, astringency sensitivity to tannic acid and saliva was assessed in 30 elderly people and 24 young people. Basic PRPs (bPRPs) and glycosylated PRPs (gPRPs) were quantified immunochemically via western blot analysis. The results showed that the amounts of bPRPs and gPRPs were similar between the young and elderly groups. However, a positive correlation between the gPRP amount and astringency threshold was observed only in the young group, while a negative correlation between the bPRP amount and astringency threshold was observed only in the elderly group. This finding suggests differences in the contribution of PRP type to astringency perception as a function of age.

1. Introduction

Ageing exerts significant impacts on various aspects of oral physiology. With advancing age, individuals may experience tooth loss, alterations in saliva production and composition, oral cancer and changes in flavour perception. These alterations arise from a multitude of factors, including genetics, lifestyle habits, medications, and health conditions (Lamster, Asadourian, Del Carmen, & Friedman, 2016; Riera & Dillin, 2016; Schwartz, Vandenberghe-Descamps, Sulmont-Rossé, Tournier, & Feron, 2018). Consequently, these changes can have detrimental effects on food intake, leading to reduced consumption of essential nutrients such as vegetables, nuts and fish (Muñoz-González, Vandenberghe-Descamps, Feron, Canon, Labouré, & Sulmont-Rossé, 2018; Vandenberghe-Descamps, Laboure, Septier, Feron, & Sulmont-Rosse, 2018). Ultimately, this can result in malnutrition and diet-related diseases, including cardiovascular disease and diabetes (Dainy, Kusharto, Madanijah, Nasrun, & Turana, 2018).

One notable consequence of decreased vegetable and fruit consumption is the reduced intake of polyphenols. Research indicates that a diet rich in polyphenols might reduce the risk of age-related diseases, such as cardiovascular diseases, diabetes and cancer (Vauzour et al., 2017; Borsoi, Neri-Numa, de Oliveira, de Araújo, & Pastore, 2023). Polyphenols encompass a diverse group of plant secondary metabolites that exhibit various chemical structures and share the common characteristic of containing multiple phenol groups. Tannins, a type of polyphenol compound, are commonly found in plant-based foods and beverages, including vegetables, nuts, unripe fruits, red wines, teas, and beers (Jiang, Gong, & Matsunami, 2014; Yang et al., 2022). Consumption of these items is frequently accompanied by sensations of drying, roughening and puckering in the mouth, referred to as astringency (ASTM, 1989).

The molecular mechanisms underlying the sensation of astringency have yet to be fully elucidated. Several hypotheses have been proposed, primarily involving the interaction of tannins with proteins. First, tannins may aggregate and precipitate salivary proteins, particularly proline-rich proteins (PRP), thereby reducing the lubricating properties of saliva. This phenomenon is perceived as increased oral friction (Soares et al., 2011; Canon et al., 2021; Guerreiro et al., 2022). Second, tannins may interact with oral epithelial cells or the salivary proteins constituting the mucosal pellicle on the oral mucosa surface (Canon et al., 2021; Ramos-Pineda, García-Estévez, Soares, de Freitas, Dueñas, & Escribano-Bailón, 2019). These interactions can decrease the lubricating properties of the mucosal pellicle or involve the transmembrane mucin MUC1 (Canon et al., 2021). According to the latter hypothesis, PRPs may prevent tannins from interacting with the oral mucosa by scavenging them, thereby reducing the perception of astringency.

* Corresponding authors.
E-mail addresses: Francis.Canon@inrae.fr (F. Canon), Gilles.Feron@inrae.fr (G. Feron).

https://doi.org/10.1016/j.fochms.2023.100192
Received 19 September 2023; Received in revised form 18 December 2023; Accepted 26 December 2023
Available online 28 December 2023

Proline-rich proteins (PRPs) constitute approximately two-thirds of the proteins secreted by human parotid glands (Pascal, Bigey, Ratomahenina, Boze, Moulin, & Sarni-Manchado, 2006). These bacteria are characterized by a high proportion of proline (25–42 %), glycine (16–22 %), and glutamic/glutamine (15–28 %) residues (Sarni-Manchado, Canals-Bosch, Mazerolles, & Cheynier, 2008). PRPs exhibit significant polymorphism and heterogeneity in terms of primary amino acid sequence, size and posttranslational modifications, which likely contribute to their functional diversity (Ramos-Pineda et al., 2019).

The high affinity between PRPs and tannins is attributed to their distinctive structural characteristics. PRPs are intrinsically disordered proteins that primarily adopt random coil structures, with small segments forming polyproline helices (Boze et al., 2010). There are three main classes of PRPs, i.e., acidic, glycosylated, and basic PRPs (Bacon & Rhodes, 2000). Acidic PRPs are known to bind calcium and inhibit crystal growth and may play a vital role in maintaining calcium homeostasis in the mouth. They are also involved in dental pellicle formation. Glycosylated PRPs contribute to oral lubrication and exhibit binding capabilities towards oral bacteria. They can also interact with tannins (Asquith, Uhlig, Mehansho, Putman, Carlson, & Butler, 1987). Basic PRPs (bPRPs) can interact with plant tannins, providing a protective mechanism against the dietary effects of these polyphenols. These interactions are also believed to be involved in the sensation of astringency in food and beverages (Pascal et al., 2006).

The capacity of bPRPs to bind and precipitate tannins has been ascribed to their proline-rich sequences and high glycine content, which confer an open structure that provides a large binding surface area and multiple contact points. IB5 is a human salivary bPRP for which a method of production by heterologous expression of the human gene PRB4S in the yeast *Pichia pastoris* and purification has been previously developed (Boze et al., 2010). Additionally, this expression system produces II-1, a glycosylated PRP, in its glycosylated (II-1) and non-glycosylated (II-1 ng) forms.

In relation to saliva and ageing, previous research has observed that changes in salivary composition are often observed with advancing age (Dodds, Johnson, & Yeh, 2005; Xu, Laguna, & Sarkar, 2019). However, the specific impact of age on the PRP concentration remains unclear. Baum et al. (1982) investigated only the level of acidic PRPs (aPRPs) in stimulated parotid glands and reported no significant correlation between the total aPRP amount or the percentage of aPRPs relative to total secretory proteins or age (Baum, Kousvelari, & Oppenheim, 1982).

In a previous study conducted by our laboratory (Wang, Septier, Brignot, Martin, Canon, & Feron, 2022), we identified differences in the oral astringency threshold based on age. Notably, we observed a correlation between salivary flow and the astringency threshold in young individuals but not in elderly individuals.

Building upon these findings, we propose the hypothesis that ageing leads to changes in the concentration of salivary PRPs, which could affect the astringency perception threshold with age. To test this hypothesis, we assessed the amount of PRP in saliva using a western blot procedure among both young and elderly participants. The aim was to examine the relationship between PRP levels and astringency sensitivity in the context of age. The results and implications of these analyses are discussed in the following sections.

2. Materials and methods

This study was approved on 31 October 2019 by the Ethical Committee CCP Ile de France IV under number 2019-A02434-53.

2.1. Panellist recruitment and sensory threshold evaluation

The detailed procedures for panel recruitment, inclusion criteria, panel training and astringency threshold evaluation have been described previously (Wang et al., 2022).

Fifty-four panellists, including 30 elderly (O) people (aged $\geq$ 65 y/o) and 24 young (Y) people (aged $\leq$ 35 y/o), were recruited to participate in the sensory sessions. The panel is described in Table 1. The elderly and young subjects had good oral health, with at least 7 functional posterior units (Vandenberghe-Descamps et al., 2016). Moreover, the elderly participants were autonomous persons living at home, had no cognitive disorders (Mini Mental State Examination (MMSE) $>$ 25 (Folstein, Folstein, & McHugh, 1975)), did not have complete or half-complete dental appliances and took an average of 2 drugs per day (median = 1).

2.1.1. Preliminary session

The objective of this session was to ensure that the participants were able to (i) clearly identify and differentiate astringency from other sensory sensations, particularly sourness, bitterness and olfactory cues, and (ii) perfectly understand the procedure of the sensory test, i.e., the 2-AFC, for use later.

The session was divided into two parts. During the first part, the subjects received tasting samples (20 mL each) in a fixed order at room temperature in plastic cups coded with random numbers. The participants were instructed to put the samples into their mouths, swirl the sample gently in the mouth for 30 s, spit it out and judge which taste it was. Between samples, the subjects rinsed their mouth with Evian water and then waited for 1 min before the next sample. The tasting sensations were saltiness, sweetness, sourness, bitterness and umami. Additionally, the panellists were presented with a tannic acid solution as an example of astringency.

In the second part, the subjects were trained and familiarized with the 2-AFC procedure as described below.

During both parts of the preliminary session, there was a discussion between the subjects and the experimenters after each test. At the end of the session, all of the panellists indicated that they were able to (i) clearly distinguish astringency from the other sensory sensations and (ii) perform the 2-AFC test properly.

2.1.2. Testing session

The astringency threshold was evaluated by a 2-AFC procedure with ascending concentrations of tannic acid. In each 2-AFC presentation, two samples were presented: a target sample and a control sample. Each 2-AFC test was performed 3 times, and the evaluation was performed 3 times in 3 different sessions. Paired samples (5 mL) were presented in a balanced order following a Latin square design (Williams design) at room temperature in a white plastic cup coded with the letter A or B. The testing procedure started from the lowest concentration. Panellists were given the reference or stimulus sample. The participants were asked to put the samples into their mouth, swirl them gently around the mouth for 30 s, and then spit them out. The panellists then rinsed their mouths and waited 1 min before the second sample was evaluated. After 30 additional seconds, the panellists were asked to indicate which sample was perceived as astringent. Then, the panellists rinsed their mouths as described previously (Wang et al., 2022).

A sensitivity level was reached when three correct answers were obtained from the same concentration. The best estimate threshold for each subject was evaluated as the geometric mean of the three correctly answered concentrations and the previous lower concentration. When the subjects correctly identified the lowest concentration (0.02 g/l), the geometric means were calculated between this concentration and the theoretical concentration below it, i.e., 0.02/3.05 = 0.0065 g/l. In contrast, when subjects did not correctly identify the highest concentration (0.574 g/l), the geometric mean was calculated between this concentration and the theoretical concentration above it, i.e., 0.574*3.05 = 1.75 g/l.

2.2. Saliva sample collection and treatment

Saliva was collected at the start and end of the 3 different sessions. Saliva collection and saliva sample treatment and storage procedures

Table 1
Characteristics of the young and elderly participants, thresholds, salivary flow (SF) and PRP amounts in the two groups.

	Y (n = 24)				O (n = 30)			
	Mean	Median	Range	SD	Mean	Median	Range	SD
Age (years)	29.4	30	24–35	3.8	75	73.5	70–87	4.23
Threshold (mg/mL)	0.29	0.2	0.04–1.00	0.26	0.41	0.35	0.06–0.78	0.24
SF (mL/min)	0.49	0.47	0.27–0.82	0.16	0.42	0.35	0.11–0.92	0.23
bPRPs Mean (μg/mL)	10.16	8.57	0.19–28.98	7.67	11.99	10.78	1.33–34.11	9.05
bPRPs Start (μg/mL)	10.20	7.18	0–29.67	8.16	12.42	10.67	1.98–31.09	8.89
bPRPs End (μg/mL)	10.11	8.77	0.23–28.29	7.53	11.56	9.42	0–37.14	9.78
gPRPs Mean (μg/mL)	352.50	217.35	51.83–1136.49	308.87	640.68	360.48	0–2299.72	659.43
gPRPs Start (μg/mL)	402.59	244.71	53.12–1242.66	397.99	819.96	472.37	0–3066.78	910.26
gPRPs End (μg/mL)	308.43	205.95	43.49–1342.03	308.23	444.79	243.98	0–1584.48	442.96

SD: standard deviation of the mean.

were performed as described previously (Wang et al., 2022). Flow rates were expressed as grams of saliva per minute (g/min). Saliva samples were dried with a SpeedVac device, and each dried saliva sample was then dissolved in 20 μL of buffer (Laemmli 2x, Bio-Rad) without a denaturing agent. Then, the Eppendorf tubes with the reconstituted saliva samples were heated in boiled water for 3 min before western blot analysis.

2.3. Western blot

2.3.1. Standard PRP production

Both the PRPs IB5 and II-1 were produced and purified in our laboratory as described previously (Boze et al., 2010), with slight modifications. Briefly, the *Pichia pastoris* GS115 strain harbouring the pPICPRB4S vector expression plasmid containing the PRB4 gene (NCBI: txid9606) was incubated in buffered glycerol complex medium (BMGY, 10 g/L glycerol) at 28 °C and 200 rpm until the optical density at 600 nm reached 40, corresponding to 20 g of dry cell weight per litre. The biomass was transferred into synthetic excretion buffered methanol complex medium (BMMY, 10 mL/L methanol). The expression and secretion of the recombinant proteins were thereafter induced by gradually adding methanol over 116 h for a total of 150 mL/L.

After centrifugation at 15000 × g for 30 min at 4 °C, the supernatant was filtered through a 0.2 μm filter and dialyzed (with a 1 kDa cut-off) against 50 mM Tris-HCl, pH 8.0, for 48 h. The proteins were loaded onto a cation exchange chromatography SP XL Streamline column (GE Healthcare, Little Chalfont, UK) in 50 mM Tris HCl (pH 8.0) with a NaCl gradient from 0 to 1 M. After pooling the fractions containing the pure protein, the samples were lyophilized and loaded onto a gel filtration HiPrep Sephacryl S100-26/60 column (GE Healthcare, Little Chalfont, United Kingdom) in 50 mM ammonium acetate. After pooling the fractions containing each pure PRP sample, the mixture was lyophilized and stored at −80 °C.

The PRPs were analysed by mass spectrometry in MS and MS2 CID (collision-induced dissociation) mode with an Orbitrap Tribrid Fusion (Thermo Fisher Scientific, Waltham, USA) after electrospray ionization (ESI) in positive mode and intact protein mode. The spectra were in accordance with previous production batch spectra (Pascal et al., 2006; Boze et al., 2010; Canon et al., 2013).

2.3.2. Antibodies against IB5

Polyclonal antibodies were designed with and purchased from Eurogentec (Seraing, Belgique). It was produced in rabbits against the full amino acid sequence of the purified recombinant proteins IB5 produced in our laboratory. Final bleed containing antibodies raised against IB5 has been validated by ELISA assay by Eurogentec.

Reactivity against pure recombinant II-1 also produced by the same *P. Pastoris* transformed strain has already been demonstrated (Brignot et al., unpublished results).

2.3.3. SDS-PAGE and western blot analyses

All of the samples collected at the beginning (B) and at the end (E) of the 3 sensory sessions were analysed (n = 6) as one replicate.

Fifty microlitres of saliva sample was concentrated until dry with an RC 10.22 Jouan SpeedVac device (Thermo Fisher Scientific, Waltham, USA). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described by Laemmli (1970) with minor modifications using 12 % TGX acrylamide precast gels and a Mini-Protean II system (Bio-Rad, Hercules, USA) at a constant voltage of 200 V for 35 min. A Kaleidoscope molecular weight ladder (Bio-Rad, Hercules, USA) was added to each gel to follow protein migration.

Briefly, proteins were separated by SDS-PAGE and transferred electrophoretically to a PVDF membrane with a TransBlot Turbo Transfer System (Bio-Rad, Hercules, USA). Thereafter, the membrane was incubated in blocking solution (8 % (w/v) skim milk and 0.9 % (w/v) NaCl solution) for 60 min with gentle shaking at room temperature. The membrane was first probed with primary antibodies against IB5 at 1:1000 at room temperature for 60 min. After 2 washes with 0.05 % (v/v) Tween 20 in PBS, the mixture was incubated again in blocking solution for 30 min under gentle shaking at room temperature. The membrane was washed with 0.9 % (w/v) NaCl and probed with secondary mouse anti-rabbit IgG HRP-conjugated (Invitrogen Thermo Fisher Scientific, USA) at 1:5000 at room temperature for 60 min. The membrane was thoroughly washed 3 times with 0.05 % (v/v) Tween 20 in PBS for 5 min. Next, the membrane was incubated with a mixture of a peroxide reagent and luminol-enhancer reagent from the Clarity™ Western ECL Substrate kit (Bio-Rad, Hercules, USA) for chemiluminescence development. The membrane was immediately scanned with a ChemiDoc imaging system (Bio-Rad, Hercules, USA) for a 90 sec exposure time with 1 sec steps. The exposure selection criterion was signal saturation. The images taken one second before target signal (from IB5 or II-1) saturation were selected for quantification.

2.3.4. PRP quantification

Western blotting was used to assess the amount of bPRPs and gPRPs in the saliva.

Recombinant IB5 and II-1 were used as references for bPRP and gPRP, respectively. II-1 was considered to be present in all possible glycosylated forms, so a target molecular weight range was selected.

The membrane area corresponding to bPRPs in saliva was determined from 10 western blots conducted on different days, and the membrane area corresponding to gPRPs was determined as described above.

The calibration ranges for IB5 and II-1 were designed to quantify the bPRPs and gPRPs in the saliva samples. To handle intermembrane variability, 3 μg/mL IB5 and 3 μg/mL II-1 were used as positive controls to normalize the intensity of the raw signals.

The signal intensities from the selected areas on the membranes of the targeted proteins bPRPs and gPRPs were compared to those of pure recombinant IB5 and II-1 from the calibration range to yield quantitative measures for bPRPs and gPRPs, respectively.

2.4. Statistical analysis

Nonparametric analyses were conducted because normality assumptions were not met. Mann-Whitney U tests were performed to evaluate differences between the Y and O groups regarding salivary PRP. Wilcoxon tests were performed on the PRP amount-to-pixel volume ratio to evaluate differences between the start and end of each session. Friedman ANOVA was also conducted on the PRP amount to evaluate differences among the three sessions. Spearman rank order correlations were performed for the whole group and for each group (Y and O) to evaluate the relationships between the astringency threshold and the PRP amount. The significance was set at $p < 0.05$. These tests were performed using Statistica® version 13.5.0.17 (1984–2018; TIBCO Software, Inc.).

3. Results

3.1. Threshold and salivary flow

In agreement with our prior investigation (Wang et al., 2022), dissimilarities were detected between the Y and O groups (Table 1). Specifically, the O group exhibited a greater mean astringency threshold than the Y group did ($Z = -2.5$, $P = 0.0110$). The salivary flow rate (SF) in the O group was lower than that in the Y group, with a modest level of statistical support ($Z = 1.66$, $p = 0.09$).

3.2. Western blot

The concentrations of the calibration range used for the quantification of bPRPs and gPRPs in the saliva samples were 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 μg/mL of pure recombinant IB5 and 0.25, 1, 3, 5, 10, 25 and 50 μg/mL of pure recombinant II-1. The selected areas are presented in Figs. 1 and 2, respectively. and ranged from 12 kDa to 18 kDa for bPRPs and from 20 kDa to 150 kDa for gPRPs.

Calibration was performed by linear regression with the following parameters for IB5: $y = 0.2885x + 0.0631$, $r^2 = 0.9939$; and for II-1: $y = 0.1812 + 0.4294$, $r^2 = 0.9984$.

Figs. 3 and 4 show representative western blot analyses from a panellist with a high amount of bPRPs but within the average range for gPRPs. The major signal at approximately 50 kDa corresponds to gPRPs, and the band at approximately 14 kDa corresponds to bPRPs.

3.3. PRP amount

No significant differences were found between sessions in terms of the quantity of bPRPs for Group Y (Friedman $Chi^2 = 1.33$, $p = 0.51$) or Group O (Friedman $Chi^2 = 1.87$, $p = 0.39$) (Table 1). Furthermore, no differences were observed between the mean values at the start and end of the sessions for the Y ($Z = 0.31$, $p = 0.75$) or O ($Z = 0.89$, $p = 0.37$) groups. Similarly, no significant difference was noted in the mean amount of bPRPs between the Y and O groups ($Z = -0.51$, $p = 0.61$). Similarly, no differences were observed between the Y or O group in terms of bPRPs Start ($Z = -0.81$, $p = 0.42$) or bPRPs End ($Z = -0.55$, $p = 0.58$).

No differences were observed between the three sessions regarding the amount of gPRPs for either the Y group ($Chi^2 = 3.58$, $p = 0.17$) or the O group ($Chi^2 = 3.29$, $p = 0.19$). However, a significant difference was detected within the O group between the start of the session (mean = 819.96 μg/mL) and the end of the session (mean = 444.79 μg/mL) in terms of gPRP quantity ($Z = 4.36$, $p = 0.000013$). A moderate difference was observed in the Y group between the session start (mean = 402.59 μg/mL) and session end (mean = 308.43 μg/mL) regarding the amount of gPRPs ($Z = 1.74$, $p = 0.08$).

However, no significant differences were found between the Y or O group in terms of gPRP quantity, whether considering the mean value ($Z = -1.30$, $p = 0.19$), the start value ($Z = -1.57$, $p = 0.12$), or the end value of the session ($Z = -0.55$, $p = 0.58$).

3.4. Correlation between astringency threshold and PRP amount

In the O group, a significant and negative correlation was observed between the mean bPRP amount and the threshold ($r = -0.41$, $p = 0.02$) (Fig. 5). This finding suggested that as the amount of bPRP increased, the astringency threshold decreased. Conversely, no correlation was found between the mean bPRP amount and the threshold in the Y group ($r = -0.16$, $p = 0.45$).

Within the Y group, a significant and positive correlation was identified between the mean gPRP amount and threshold ($r = 0.49$, $p = 0.01$). This observation indicated that as the gPRP amount increased, the threshold of astringency perception also increased (Fig. 6). Similarly, significant and positive correlations were observed between the gPRPs Start ($r = 0.46$, $p = 0.02$) and gPRPs End ($r = 0.53$, $p = 0.007$) and the threshold. However, no significant correlation was found between the mean gPRP amount and the threshold in the O group ($r = 0.05$, $p = 0.79$). Likewise, no significant correlations were observed between the gPRPs Start ($r = 0.04$, $p = 0.83$) or gPRPs End ($r = -0.04$, $p = 0.81$) and the threshold in the O group.

4. Discussion

Astringency is a multifaceted sensation characterized by oral surface sensations of drying, roughing and puckering of the mucosa surrounding the mouth (Horne, Hayes, & Lawless, 2002). Despite its significance in food flavour perception, few studies have examined the age-related variation in astringency sensitivity. In our previous study, we identified differences in oral astringency perception based on age, which was not attributed to variations in salivary flow rates between the groups (Wang et al., 2022). Consequently, the present study aimed to elucidate

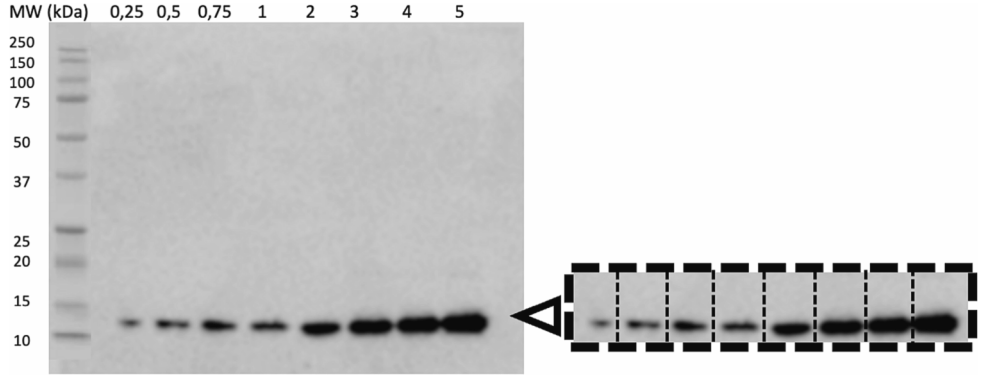

Fig. 1. Membrane image of IB5 gradient concentrations. The standard range of IB5 is expressed in μg/mL.

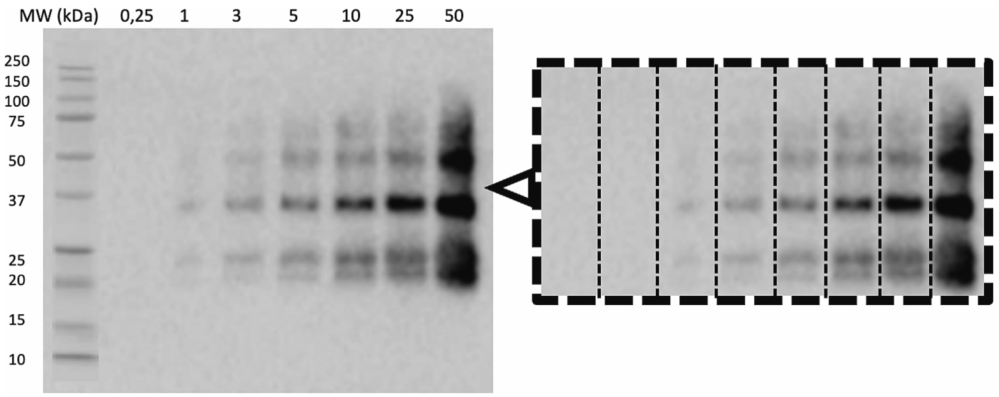

Fig. 2. Membrane image of Il-1 gradient concentrations. The standard range of II-1 is expressed in μg/mL.

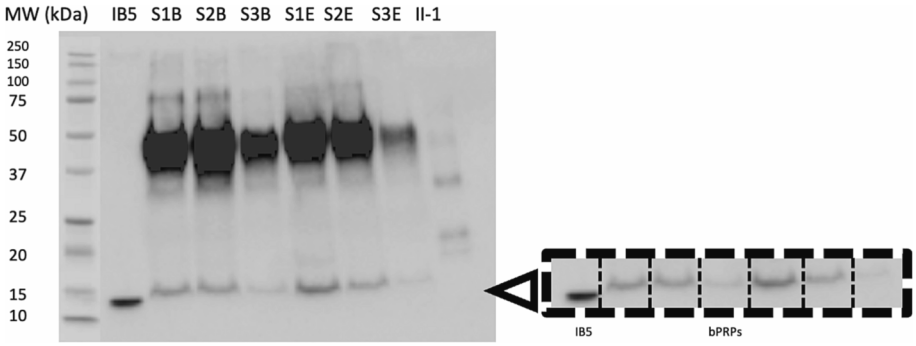

Fig. 3. Detection and quantification of bPRPs in the panelists' samples. The membrane exposure time was 6 sec. IB5 and II-1 were incubated at 3 μg/mL. S1, S2, and S3 correspond to 3 different sessions conducted on different days, and sampling was performed at the beginning (B) or at the end (E) of the sessions. The arrow represents the targeted bPRPs and the dotted outline represents the selected area.

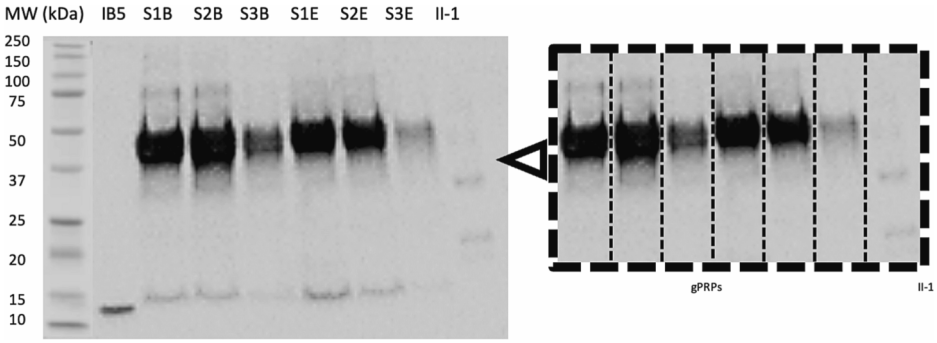

Fig. 4. Detection and quantification of gPRPs in the panelists' samples. The membrane exposure time was 1 sec. IB5 and II-1 were used at 3 μg/mL. S1, S2, and S3 correspond to 3 different sessions conducted on different days, and sampling was performed at the beginning (B) or at the end (E) of the sessions. The arrows represent the targeted gPRPs, and the dotted outline represents the selected area.

these differences by exploring the salivary composition, particularly the protein patterns and, specifically, the abundance of PRPs.

First, the present study highlighted the large interindividual variability in both the young and elderly panels regarding sensitivity to astringency and salivary parameters. It is well known that taste responsiveness can vary substantially among individuals. Among other factors, this variation comes from genetic differences, sex, taste pathologies, age, BMI, and ethnic background (Prescott & Tepper, 2004; Williams, Bartoshuk, Fillingim, & Dotson, 2016). Large interindividual differences in salivary flow and composition have also been repeatedly reported in the literature. Different types of factors can explain this variation, such as lifestyle factors (diet and smoking habits, for instance), age, sex and ethnicity (Mosca, Stieger, Neyraud, Brignot, van de Wiel, & Chen, 2019; Quintana et al., 2009; Vandenberghe-Descamps et al., 2016).

Assessing the PRP level in saliva, we found no disparities in either the bPRP or gPRP amount between the young and elderly participants. Baum et al. previously reported unaltered aPRP quantity with age (Baum et al., 1982). Thus, the PRP concentration in saliva is not dependent on age. Age-related changes in salivary protein concentrations seem to exhibit an age-dependent influence on specific components and distinct secretory glands. For instance, it is widely acknowledged that mucin levels decline with age in unstimulated whole saliva; stimulated submandibular/sublingual saliva; and mucous glands, such as the submandibular, sublingual, and minor salivary glands (Arjan Vissink, 1996; Dodds et al., 2005; Xu et al., 2019).

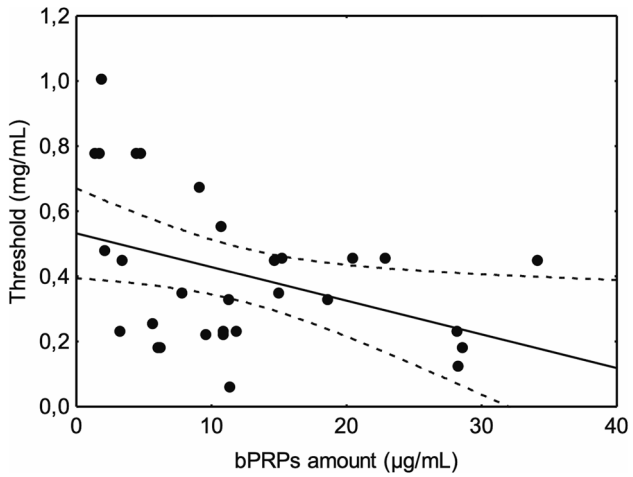

Fig. 5. Spearman correlation between astringency threshold and the mean bPRP amount observed in the group of elderly panellists. The solid line corresponds to the fitted data. The dotted line corresponds to the confidence interval at 95%. The black dots represent individual data points.

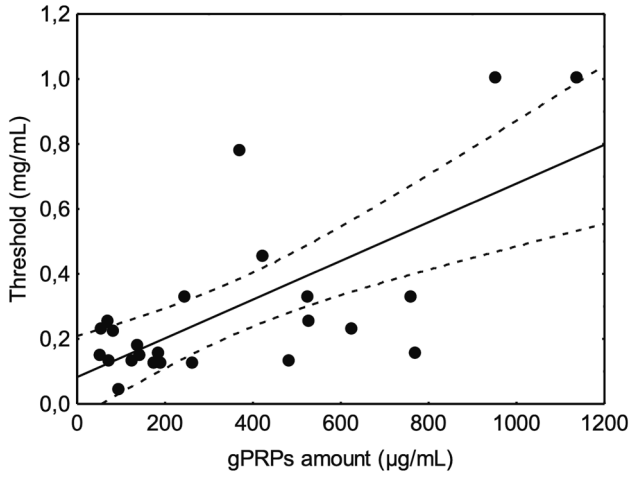

Fig. 6. Spearman correlation between astringency threshold and the mean gPRPs observed in the group of young panellists. The solid line corresponds to the fitted data. The dotted line corresponds to the confidence interval at 95%. The black dots represent individual data points.

Interestingly, we observed a decrease in the quantity of gPRPs at the end of the session compared to the initial level, especially in the elderly group. The available literature on gPRP concentrations in saliva, particularly before and after sensory testing, is limited. Speculatively, this observation may be attributed to the multiple rinsing procedures occurring during the sensory test, which could dilute saliva or remove the salivary film, consequently reducing the concentration of gPRPs in saliva. Notably, alterations in the salivary film have been suggested to influence its rheological and lubricating properties, and gPRPs are recognized as contributors to oral lubrication (Gibbins & Carpenter, 2013). Furthermore, rinsing the mouth with water significantly reduces protein levels in saliva (Nayak & Carpenter, 2008). Our findings suggest that this effect is more pronounced in elderly participants. Additionally, the saliva of elderly individuals may exhibit a lower recovery of its normal composition than that of young individuals following external interventions. Indeed, the ability of individuals to maintain constant saliva characteristics is thought to modulate astringency sensitivity (Dinnella, Recchia, Fia, Bertuccioli, & Monteleone, 2009).

Upon examining the elderly group, we observed a significant negative correlation between bPRPs and the astringency threshold, indicating that increased bPRP levels were associated with heightened sensitivity to astringent compounds. These findings suggested that bPRP plays a direct role in the detection of astringent compounds. bPRP has been shown to effectively scavenge tannins (Canon, Giuliani, Paté, & Sarni-Manchado, 2010), forming soluble noncovalent complexes (Canon et al., 2009) that subsequently aggregate and precipitate (Canon et al., 2013). The aggregation of bPRP and tannins involves the binding of tannins to two proteins simultaneously, forming a bridge between them. Notably, a study of the interactions between bPRP and IB5 and between flavan-3-ol and epigallocatechin gallate (EgCG) revealed that three EgCG proteins are needed to form aggregates (Canon et al., 2013). The strong affinity of bPRP for tannins is attributed to their unstructured conformation, which allows them to undergo a structural transition from an unfolded to a folded state upon tannin binding (Canon et al., 2011). These structural rearrangements involve flexible amino acids surrounding rigid clusters of polyproline residues (Canon et al., 2015), which serve as preferential binding sites for tannins (Canon et al., 2013) and allow the establishment of additional hydrogen bonds (Canon et al., 2010). Previous studies have shown that the precipitation threshold of bPRP, IB5, with EgCG is similar to the astringency threshold of the compound (Canon et al., 2013). These observations suggest that the aggregation of bPRP by tannins may be the mechanism underlying astringency perception.

Conversely, in the young group, a significant and positive correlation was found between gPRPs and the astringency threshold. This implies that higher amounts of gPRPs are associated with higher thresholds for astringency. Hence, we speculate that gPRP plays a protective role by binding tannins before they interact with the mucosal pellicle. This mechanism has been previously reported *in vitro* for bPRP IB5, which protects the oral mucosa from tannin-induced mucosal pellicle aggregation (Ployon et al., 2018). The discrepancy observed between bPRP and gPRP may be attributed to the lower sensitivity of human gPRP to tannin-induced aggregation than bPRP. This phenomenon is likely due to steric hindrance caused by the presence of carbohydrates on the peptide chain of gPRPs, which may prevent tannins from bridging two proteins. Moreover, the negative charge on the carbohydrate residues may maintain proteins in relatively open conformations and keep them apart through repulsion charges. Soluble tannin-protein complexes likely play a significant role in scavenging, thereby mitigating the antinutritional effects of dietary tannins.

5. Conclusion

Our findings shed light on the relationship between PRP levels and astringency perception, particularly in the context of age differences. The observed disparities in astringency perceptions between the young and elderly groups cannot be solely attributed to variations in the abundances of the two groups of PRPs (bPRPs and gPRPs). Nevertheless, within each group, significant correlations were observed between PRP levels and the astringency threshold. In the elderly group, basic PRP levels exhibited a negative correlation with the astringency threshold. This finding suggested the involvement of PRPs in astringency detection through precipitation at the surface of the oral mucosa. Conversely, in the young group, glycosylated PRP levels were positively correlated with the astringency threshold, suggesting that they play a protective role in preventing tannin-mucosal pellicle interactions.

These findings contribute to our understanding of the mechanisms underlying astringency perception and the potential modulation of this perception by PRPs in saliva. However, further analysis should encompass comprehensive analyses that consider other salivary factors, including other tannin binding proteins, such as statherin and histatins (Soares et al., 2011). This broader exploration has the potential to provide a deeper understanding of the molecular mechanisms underlying astringency perception.

All the authors have read and agreed to the published version of the manuscript.

Ethical statement

This study was approved on 31 October 2019 by the Ethical Committee CCP Ile de France IV under number 2019-A02434-53.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to publish the results.

CRediT authorship contribution statement

Mei Wang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Hélène Brignot:** Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Writing - original draft. **Chantal Septier:** Methodology, Investigation, Resources. **Christophe Martin:** Methodology, Resources. **Francis Canon:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Visualization, Supervision. **Gilles Feron:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the Chemosens platform for providing all facilities for performing the sensory studies.

Funding sources

Mei Wang received scholarship funding from the China Scholarship Council (China) (CSC No. 201808330410). This work was supported by grants from the GIRACT Foundation (Switzerland), the Conseil Régional Bourgogne, Franche-Comte (PARI grant) and the FEDER (European Funding for Regional Economic Development).

References

Arjan Vissink, D. (1996). PhD, Frederik Karst Lucien Spijkervet, DDS, PhD, Arie Van Nieuw Amerongen, PhD. *Aging and saliva: A review of the literature SCD Special Care in Dentistry, 16*(3), 95–103.

Asquith, T. N., Uhlig, J., Mehansho, H., Putman, L., Carlson, D. M., & Butler, L. (1987). Binding of condensed tannins to salivary proline-rich glycoproteins - the role of carbohydrate. *Journal of Agricultural and Food Chemistry, 35*(3), 331–334. https://doi.org/10.1021/jf00075a012

Astm. (1989). *Standard definitions of terms relating to sensory evaluation of materials and products. In Annual Book of ASTM Standards* (p. 2). Philadelphia: American Society of Testing and Materials.

Bacon, J. R., & Rhodes, M. J. C. (2000). Binding affinity of hydrolyzable tannins to parotid saliva and to proline-rich proteins derived from it. *Journal of Agricultural and Food Chemistry, 48*(3), 838–843. https://doi.org/10.1021/jf990820z

Baum, B. J., Kousvelari, E. E., & Oppenheim, F. G. (1982). Exocrine protein secretion from human-parotid glands during aging - stable release of the acidic proline-rich proteins. *Journals of Gerontology, 37*(4), 392–395. https://doi.org/10.1093/geronj/37.4.392

Borsoi, F. T., Neri-Numa, I. A., de Oliveira, W. Q., de Araújo, F. F., & Pastore, G. M. (2023). Dietary polyphenols and their relationship to the modulation of non-communicable chronic diseases and epigenetic mechanisms: A mini-review. *Food Chemistry: Molecular Sciences, 6*.

Boze, H., Marlin, T., Durand, D., Pérez, J., Vernhet, A., Canon, F., … Cabane, B. (2010). Proline-rich salivary proteins have extended conformations. *Biophysical Journal, 99*, 656–665. https://doi.org/10.1016/j.bpj.2010.04.050

Canon, F., Paté, F., Meudec, E., Marlin, T., Cheynier, V., Giuliani, A., & Sarni-Manchado, P. (2009). Characterization, stoichiometry and stability of salivary protein-tannin complexes by ESI-MS and ESI-MS/MS. *Analytical and Bioanalytical Chemistry, 395*(8), 2535–2545.

Canon, F., Giuliani, A., Paté, F., & Sarni-Manchado, P. (2010). Ability of a salivary intrinsically unstructured protein to bind different tannin targets revealed by mass spectrometry. *Analytical and Bioanalytical Chemistry, 398*, 815–822. https://doi.org/10.1007/s00216-010-3997-9

Canon, F., Ballivian, R., Chirot, F., Antoine, R., Sarni-Manchado, P., Lemoine, J., r., m.,, &, Dugourd,, & P.. (2011). Folding of a Salivary Intrinsically Disordered Protein upon Binding to Tannins. *Journal of the American Chemical Society, 133*(20), 7847–7852. https://doi.org/10.1021/ja200534f

Canon, F., Paté, F., Cheynier, V., Sarni-Manchado, P., Giuliani, A., Pérez, J., … Cabane, B. (2013). Aggregation of the Salivary Proline-Rich Protein IB5 in the Presence of the Tannin EgCG. *Langmuir, 29*(6), 1926–1937. https://doi.org/10.1021/la3041715

Canon, F., Ployon, S., Mazauric, J.-P., Sarni-Manchado, P., Réfrégiers, M., Giuliani, A., & Cheynier, V. (2015). Binding site of different tannins on a human salivary proline-rich protein evidenced by dissociative photoionization tandem mass spectrometry. *Tetrahedron, 71*(20), 3039–3044. https://doi.org/10.1016/j.tet.2014.11.013

Canon, F., Belloir, C., Bourillot, E., Brignot, H., Briand, L., Feron, G., … Neiers, F. (2021). Perspectives on Astringency Sensation: An Alternative Hypothesis on the Molecular Origin of Astringency. *Journal of Agricultural and Food Chemistry, 69*(13), 3822–3826. https://doi.org/10.1021/acs.jafc.0c07474

Dainy, N. C., Kusharto, C. M., Madanijah, S., Nasrun, M. W. S., & Turana, Y. (2018). Nutritional Status, Physical Activity, Oxidative Stress, and Cognitive Function in Pre Elderly and Elderly. *Jurnal Gizi Dan Pangan, 13*(3), 117–122. https://doi.org/10.25182/jgp.2018.13.3.117-122

Dinnella, C., Recchia, A., Fia, G., Bertuccioli, M., & Monteleone, E. (2009). Saliva Characteristics and Individual Sensitivity to Phenolic Astringent Stimuli. *Chemical Senses, 34*(4), 295–304. https://doi.org/10.1093/chemse/bjp003

Dodds, M. W. J., Johnson, D. A., & Yeh, C. K. (2005). Health benefits of saliva: A review. *Journal of Dentistry, 33*(3), 223–233. https://doi.org/10.1016/j.jdent.2004.10.009

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12*(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Guerreiro, C., Brandão, E., de Jesus, M., Gonçalves, L., Pérez-Gregório, R., Mateus, N., … Soares, S. (2022). New insights into the oral interactions of different families of phenolic compounds: Deepening the astringency mouthfeels. *Food Chemistry, 375*, Article 131642. https://doi.org/10.1016/j.foodchem.2021.131642

Gibbins, H. L., & Carpenter, G. H. (2013). Alternative Mechanisms of Astringency - What Is the Role of Saliva? *Journal of Texture Studies, 44*(5), 364–375. https://doi.org/10.1111/jtxs.12022

Horne, J., Hayes, J., & Lawless, H. T. (2002). Turbidity as a measure of salivary protein reactions with astringent substances. *Chemical Senses, 27*(7), 653–659. https://doi.org/10.1093/chemse/27.7.653

Jiang, Y., Gong, N. H. N., & Matsunami, H. (2014). Astringency: A More Stringent Definition. *Chemical Senses, 39*(6), 467–469. https://doi.org/10.1093/chemse/bju021

Lamster, I. B., Asadourian, L., Del Carmen, T., & Friedman, P. K. (2016). The aging mouth: differentiating normal aging from disease. Periodontology 2000, 72(1), 96-107. https://doi.org/https://doi.org/10.1111/prd.12131.

Mosca, A. C., Stieger, M., Neyraud, E., Brignot, H., van de Wiel, A., & Chen, J. (2019). How are macronutrient intake, BMI, ethnicity, age, and gender related to the composition of unstimulated saliva? A case study. *Journal of Texture Studies, 50*(1), 53–61. https://doi.org/10.1111/jtxs.12362

Muñoz-González, C., Vandenberghe-Descamps, M., Feron, G., Canon, F., Labouré, H., & Sulmont-Rossé, C. (2018). Association between salivary hypofunction and food consumption in the elderlies. A systematic literature review. *The journal of nutrition, health & aging, 22*(3), 407–419. https://doi.org/10.1007/s12603-017-0960-x

Nayak, A., & Carpenter, G. H. (2008). A physiological model of tea-induced astringency. *Physiology and Behavior, 95*(3), 290–294. https://doi.org/10.1016/j.physbeh.2008.05.023

Pascal, C., Bigey, F., Ratomahenina, R., Boze, H., Moulin, G., & Sarni-Manchado, P. (2006). Overexpression and characterization of two human salivary proline rich proteins. *Protein Expression and Purification, 47*(2), 524–532. https://doi.org/10.1016/j.pep.2006.01.012

Ployon, S., Morzel, M., Belloir, C., Bonnotte, A., Bourillot, E., Briand, L., … Canon, F. (2018). Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of bPRPs. *Food chemistry, 253*, 79–87.

Prescott, J., & Tepper, B. J. (2004). *Genetic variation in taste sensitivity*. CRC Press.

Quintana, M., Palicki, O., Lucchi, G., Ducoroy, P., Chambon, C., Salles, C., & Morzel, M. (2009). Inter-individual variability of protein patterns in saliva of healthy adults. *Journal of Proteomics, 72*(5), 822–830. https://doi.org/10.1016/j.jprot.2009.05.004

Ramos-Pineda, A. M., García-Estévez, I., Soares, S., de Freitas, V., Dueñas, M., & Escribano-Bailón, M. T. (2019). Synergistic effect of mixture of two proline-rich-protein salivary families (aPRP and bPRP) on the interaction with wine flavanols. *Food Chemistry, 272*, 210–215. https://doi.org/10.1016/j.foodchem.2018.08.024

Riera, C. E., & Dillin, A. (2016). Emerging role of sensory perception in aging and metabolism. *Trends in Endocrinology and Metabolism, 27*(5), 294–303.

Sarni-Manchado, P., Canals-Bosch, J. M., Mazerolles, G., & Cheynier, V. (2008). Influence of the Glycosylation of Human Salivary Proline-Rich Proteins on Their Interactions with Condensed Tannins. *Journal of Agricultural and Food Chemistry, 56* (20), 9563–9569. https://doi.org/10.1021/jf801249e

Soares, S., Vitorino, R., Osório, H., Fernandes, A., Venâncio, A., Mateus, N., … de Freitas, V. (2011). Reactivity of Human Salivary Proteins Families Toward Food Polyphenols. *Journal of Agricultural and Food Chemistry, 59*(10), 5535–5547. https://doi.org/10.1021/jf104975d

Schwartz, C., Vandenberghe-Descamps, M., Sulmont-Rossé, C., Tournier, C., & Feron, G. (2018). Behavioral and physiological determinants of food choice and consumption at sensitive periods of the life span, a focus on infants and elderly. *Innovative Food Science & Emerging Technologies, 46*(April), 91–106. https://doi.org/10.1016/j.ifset.2017.09.008

Vandenberghe-Descamps, M., Labouré, H., Prot, A., Septier, C., Tournier, C., Feron, G., & Sulmont-Rossé, C. (2016). Salivary flow decreases in healthy elderly people independently of dental status and drug intake. *Journal of Texture Studies, 47*, 353–360.

Vandenberghe-Descamps, M., Laboure, H., Septier, C., Feron, G., & Sulmont-Rosse, C. (2018). Oral comfort: A new concept to understand elderly people's expectations in terms of food sensory characteristics. *Food Quality and Preference, 70*, 57–67. https://doi.org/10.1016/j.foodqual.2017.08.009

Vauzour, D., Camprubi-Robles, M., Miquel-Kergoat, S., Andres-Lacueva, C., Banati, D., Barberger-Gateau, P., … Ramirez, M. (2017). Nutrition for the ageing brain: Towards evidence for an optimal diet. *Ageing Research Reviews, 35*, 222–240. https://doi.org/10.1016/j.arr.2016.09.010

Wang, M., Septier, C., Brignot, H., Martin, C., Canon, F., & Feron, G. (2022). Astringency Sensitivity to Tannic Acid: Effect of Ageing and Saliva. *Molecules, 27*(5). https://doi.org/10.3390/molecules27051617

Williams, J. A., Bartoshuk, L. M., Fillingim, R. B., & Dotson, C. D. (2016). Exploring Ethnic Differences in Taste Perception. *Chemical Senses, 41*(5), 449–456. https://doi.org/10.1093/chemse/bjw021

Xu, F., Laguna, L., & Sarkar, A. (2019). Aging-related changes in quantity and quality of saliva: Where do we stand in our understanding? *Journal of Texture Studies, 50*(1), 27–35. https://doi.org/10.1111/jtxs.12356

Yang, J., Zhou, H., Liu, Y., Wang, H., Xu, Y., Huang, J., & Lei, P. (2022). Chemical constituents of green teas processed from albino tea cultivars with white and yellow shoots. *Food Chemistry: Molecular Sciences, 5*, Article 100143. https://doi.org/10.1016/j.fochms.2022.100143